MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

Capstone for Impact Submission | GY2019

Project Title: CSF H3F3A K27M circulating tumor DNA copy number quantifies tumor growth and in vitro treatment response

Student Name(s): Stallard, Stefanie

Advisor Names(s): Carl Koschmann

Branch: Procedure Based Care

Path of Excellence: N/A

Handover/Transition:
If this project can be continued by another UMMS student, you may contact them at the following email address/phone number (N/A if project cannot be handed over): **N/A**

Summary:

Primary brain tumors and CNS metastases shed circulating tumor DNA (ctDNA) into the CSF, which can be assessed for tumor-associated mutations. Thus far, there have been no extensive studies using droplet digital PCR (ddPCR) to detect and quantify ctDNA in the CSF of pediatric high-grade brain tumor patients. [No longer true; this is from an old abstract.]

There are also gaps in our knowledge, including the potential dependence of ctDNA amount on location of sample collection and whether ctDNA can be used to quantify tumor growth and treatment response. To address these questions, we developed a novel H3F3A K27M ddPCR assay and applied it to four pediatric patients with H3F3A K27M-mutant DIPG and GBM.

We found that ddPCR was able to detect the K27M mutation in patient CSF and that the closest relation emerged between mutant K27M copies per ng of total DNA (henceforth K27M copies) and contrast-enhancing tumor area on MRI. Multi-focal CSF sampling at autopsy of a DIPG patient exhibited differences in K27M copies by proximity to the tumor. To better understand changes in K27M copies in response to both growth and treatment of DIPG, we developed an in vitro system comprised of astrocytes (NHAs) co-cultured with luciferase-expressing human DIPG cell line DIPG007 as a means to simulate ctDNA release into the CSF.

We found that DIPG007 cells released ctDNA into culture media in proportion to their proliferation, even when the media was changed frequently to approximate the constant production and resorption of CSF. Irradiation with 8 Gy resulted in a spike in mutant ctDNA 72-120 hours post-radiotherapy before decreasing.

In summary, our study suggests that H3F3A K27M copies in the CSF of children with high-grade brain tumors have a linear relation with contrast-enhancing tumor area and that ddPCR can be used to follow treatment response including ctDNA release shortly after effective therapies.

Methodology:

Designed multiple ddPCR assays (here only focusing on H3F3A K27M assay) which allow for detection and quantification of specific genetic targets, isolated DNA from tumor tissue and CSF samples from multiple sources (intraoperative ventricular, VP shunt reservoir, lumbar puncture, cisterna magna puncture at autopsy) from pediatric brain tumor patients, utilized aforementioned assays on these samples to assess K27M mutant content, mathematically converted ddPCR results to more meaningful metrics - namely copies of mutated sequence per ng of total DNA isolated, measured contrast-enhancing tumor area on axial post-contrast T1-weighted images at cut with greatest tumor content, developed cell co-culture model using K27M-mutant human tumor cell line (DIPG007) and "normal" (but immortalized) human astrocytes (NHA), irradiated experimental condition using 8 Gy, removed and replenished cell culture media at regular intervals as surrogate for CSF, monitored cell growth throughout via continuous cell imaging, assessed for proportion of remaining viable cells comprised of DIPG007 population with luciferin-based assay, ran ddPCR on media samples.

Results/Conclusion:

While not significant, there was a trend toward increasing K27M copies in CSF with increasing contrast-enhancing tumor area (NOT tumor area overall).

The amount of K27M copies varies depending on where the CSF is collected from. It initially appeared that the amount of copies increased with CSF sampling from regions with increasing proximity to tumor, but subsequent experiments have suggested that things are not this simple.

Amount of K27M copies in culture media increases with increasing number of viable K27M-mutant cells, even when media is removed and replenished regularly (so not just from accumulation over time.)

Irradiation caused a reproducible increase in K27M copies in culture media 72-120 hours post-radiation treatment; copies then started to taper off.

Our study suggests that H3F3A K27M copies in the CSF of children with high-grade brain tumors have a linear relation with contrast-enhancing tumor area and that ddPCR can be used to follow treatment response including ctDNA release shortly after effective therapies.

Reflection/Lessons Learned:

ALWAYS assume that troubleshooting will take way more time than you think.

Don't tackle too many things at the same time; you'll make far less progress than you would focusing on a limited number.

Be willing to try something that seems ridiculous/inconsequential if you're truly stuck and can think of no other logical options.

Patient families can amaze you with their overwhelming drive to find some meaning in otherwise horrendous and unimaginable circumstances.